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(−)-Epigallocatechin-3-gallate induces up-regulation of Th1 and Th2 cytokine genes in Jurkat T cells

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ABSTRACT

In the present study, we found that (−)-epigallocatechin-3-gallate (EGCG) significantly up-regulated the mRNA expression of the Th1/Th2 cytokines including IL-2, IFN-γ, IL-5 and IL-13 in Jurkat T cells. The EGCG-induced mRNA up-regulation of IL-2 and IL-5 was predominantly affected by the extracellular signal-regulated protein kinase (ERK) signalling, whereas IL-13 gene expression, the most responsive to the EGCG treatment, was dependent on neither ERK nor c-jun NH_2-terminal kinase (JNK) signalling. IFN-γ gene expression was partially mitigated by both inhibitors of the ERK and JNK pathways. Furthermore, catalase significantly attenuated the intracellular peroxide production, phosphorylation of ERK and JNK, and all cytokine gene expressions induced by EGCG. In addition, physiologically relevant concentrations of both EGCG and H_2O_2-induced up-regulation of IL-5 gene expression. Our findings provide biological evidence that EGCG induces Th1/Th2 cytokine mRNA expression *via* H_2O_2 production followed by activation of ERK or JNK in Jurkat T cells.

Introduction

T helper 1 (Th1)[1] and Th2 subsets distinguished by the cytokines they produce have been implicated in the regulation of various immune responses [1]. Th1 cells predominantly secrete IFN-γ, IL-2 and lymphotoxin, which help to eradicate intracellular pathogens and, when overproduced, can cause autoimmunity. Th2 cells primarily produce IL-4, IL-5, and IL-13, all of which are critical for the suppression of infections with extracellular pathogens, such as parasitic worms, and when overproduced, promote the development of allergic diseases, such as atopic asthma, hay fever, and eczemas [2].

The activation of naive T helper cells is a complex intracellular process involving the ligation of multiple cell surface receptors and multiple intracellular signalling pathways. Reactive oxygen species (ROS) act as regulators of the signal transduction by cell surface receptors of T cells in the immune system [3]. ROS also play an important role in the pathogenesis of airway inflammation and hyperresponsiveness [4,5]. Therefore, oxidative stress and its modulation may influence the development of the naive T helper cell differentiation to Th1 or Th2 cells.

Green tea is the most popular and widely consumed beverage in the world, after water, in which epigallocatechin-3-gallate (EGCG) is the most abundant and active constituent [6]. EGCG is well known as a scavenger of reactive oxygen species (ROS) [7]. Conversely, EGCG also has pro-oxidant functions and is reported to induce oxidative damage, subsequent gene expression and cell apoptosis, which have been attributed to the spontaneous generation of H_2O_2 upon dissolving EGCG [8,9]. Concerning the pro-oxidant property of EGCG, knowledge about its regulatory effect on immune response has been limited. The aim of the present study was to investigate the pro-oxidant effect of EGCG on Th1 and Th2 cytokine response in Jurkat T cells, and elucidate the fundamental mechanism involved in these effects. We found that the EGCG-generated H_2O_2 contributed to the up-regulation of the tested cytokines including IL-2, IL-5, IFN-γ and IL-13. ERK regulated the gene expression of IL-2, IFN-γ, and IL-5, but not IL-13, whereas only the IFN-γ gene expression induced by EGCG was dependent on the JNK signalling pathway in Jurkat T cells.

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[1] *Abbreviations used:* EGCG, (−)-epigallocatechin-3-gallate; Th1, T helper 1; Th2, T helper 2; IL, Interleukin; IFN-γ, Interferon γ; FBS, fetal bovine serum; WST-1 reagent, tetrazolium salt; PBS(-), phosphate-buffered saline without Ca^{2+} and Mg^{2+}; MAPKs, mitogen-activated protein kinases; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated protein kinase; ROS, reactive oxygen species; H_2DCF-DA, 2′,7′-dichlorofluorescein diacetate; DCF, 2′,7′-dichlorofluorescein.

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Materials and methods

Chemicals and reagents

EGCG and WST-1 were purchased from Wako Pure Chemical Industries (Osaka, Japan); RPMI-1640 medium and fetal bovine serum were purchased from Gibco-Invitrogen (CarIsbad, CA); Catalase from bovine liver, protease and phosphatase inhibitor cocktails, H_2DCF-DA, SP600125, PD98059 were obtained from Sigma (St., Louis, MO); Antibodies to phospho-ERK, phospho-JNK, phospho-p38, JNK, and p-38 were purchased from Cell Signaling Technology (Beverly, MA); Antibodies to ERK and actin were purchased from Santa Cruz Biotechnology (CA, USA).

Cell culture

Human Jurkat T cell line (RIKEN Cell Bank; Tsukuba, Ibaraki, Japan) was maintained in RPMI-1640 medium and supplemented with 10% (v/v) FBS, 50 U/ml of penicillin and 50 μg/ml of streptomycin at 37 °C under 5% CO_2 and 95% air. For experiments, cells were seeded in complete medium and treated with each reagent or vehicle (final 0.1%, v/v).

WST-1 assay

Cell viability was evaluated using a colorimetric assay with the tetrazolium salt WST-1. Jurkat cells (2.5×10^4) were added with EGCG at various concentrations, seeded into 96-well plate and incubated for indicated periods. After treatment 10 μl of WST-1 was added to all wells of an assay, and plates were incubated at 37 °C under 5% CO_2 for 2 h. Cell viability was calculated according to the absorbance at 450 nm in a microplate reader.

H_2DCF-DA assay

Intracellular peroxide level was detected by 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) as an intracellular fluorescence probe [10,11]. Jurkat cells (2×10^6) were pre-treated with H_2DCF-DA (50 μM) for 30 min at 37 °C, and treated with EGCG (100 μM) in the presence or absence of catalase (1000 U/ml) or H_2O_2 at the various concentrations for 15 min. The cells were harvested and washed with PBS (−) twice. A flow cytometer (COULTER EPICS XL, Beckman) was used to detect DCF. Data were collected and analyzed by Win MDI 2.9 Software Program.

RT-PCR analysis

After treatments, Jurkat cells (5×10^6) were washed with ice-cold PBS (−). Total cellular RNA was isolated using the Trizol reagent (Invitrogen) according to the manufacturer's recommendations. Total RNA (8 μg) was reverse transcribed with OligodT to cDNA using M-MLV reverse transcriptase (Takara, Kyoto, Japan). PCR amplification was then performed with Taq polymerase (Takara) and specific primers. The forward and reverse primers and the expected PCR product sizes are as follows: IL-2 5′-GCCACAGAACTG AAACATCT-3′ and 5′-AGTCAGTGTTGAGATGATGC-3′ (255 bp); IFN-γ 5′-GGTCATTCAGATGTAGCGGA-3′ and 5′-GCGTTGGACATT CAAGT CAG-3' (270 bp); IL-5 5′-GCTTCTGCATTTGAGTTTGCTAGCT-3′ and 5′-TGGCCGTCAATGTATTTCTTTATTAAG-3′ (294 bp); IL-13 5′-TTG ACCACGGTCATTGCTCT-3′ and 5′-TCGATTTTGGTGTCTCGGACA-3′ (317 bp); β-actin 5′-GTCACCCACACTGTGCCCATCTA-3′ and 5′-GC AATGCCAGGGTACATGGTGGT-3′ (455 bp). The annealing temperature used in this study was 58 °C for IL-2 and IFN-γ, 60 °C for IL-5, 56 °C for IL-13 and 59 °C for β-actin. The PCR products were then subjected to agarose gel electrophoresis (2%), stained with ethidium bromide, and photographed. Densitometric analysis of the bands was carried out using the MultiGauge Software Program.

Western blotting

After treatments, Jurkat cells (2×10^6) were washed with ice-cold PBS (−), whole cell lysates were prepared in lysis buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 2 mM DTT, 10 mM NaF, 1 mM Na_3VO_4, 1 mM PMSF 1% SDS and 1% Triton X100) containing protease and phosphatase inhibitor cocktails (Sigma) and left on ice for 20 min. After sonication lysates were clarified by centrifugation at 12000 rpm for 25 min. Protein concentration was determined by Bio-Rad protein assay (Nippon Bio-Rad, Tokyo, Japan). Equal quantities of protein were subjected to SDS–PAGE and transferred to Immobilon-P membranes (Millipore). The membranes were blocked and then incubated with the primary antibody overnight at 4 °C followed by an appropriate secondary antibody. Secondary antibody binding was visualized using a Chemi-Lumi One Reagent (Nacalai Tesque, Kyoto, Japan). Densitometric analysis of the bands was carried out using the MultiGauge Software Program.

Statistical analysis

All values were expressed as means ± SD. Statistical analysis was performed by using the Student's *t*-test compared to control or between the indicated groups. A level of $P < 0.05$ was considered significant in all statistical tests.

Results

EGCG enhances intracellular peroxide level in Jurkat T cells

We investigated the effect of EGCG on the intracellular peroxide level in Jurkat T cells using H_2DCF-DA. As shown in Fig. 1A, the treatment with EGCG (100 μM) for 15 min significantly increased the intracellular peroxide level (1.3-fold). Catalase, which catalyzes the conversion of H_2O_2 into water and oxygen, completely suppressed the EGCG-induced DCF oxidation. For comparison, the same studies were performed with exogenous hydrogen peroxide (Fig. 1B). An apparent increase in the DCF fluorescence was observed in Jurkat T cells incubated with H_2O_2 at concentrations higher than 50 μM. These results implied that the EGCG-enhanced intracellular peroxide level was mainly ascribed to the extracellular H_2O_2 production. Also, the intracellular peroxide level induced by 100 μM of EGCG was similar to that triggered by 50–100 μM of exogenous hydrogen peroxide in Jurkat T cells.

EGCG and H_2O_2 up-regulate Th1 and Th2 cytokine gene expressions in Jurkat T cells

We investigated the cytotoxic effects of EGCG and H_2O_2 at the concentration of 100 μM, which was required for significant enhancement of intracellular ROS level in a DCF assay, and found that treatment with EGCG for 1 h or H_2O_2 for 30 min showed no significant effect on cell viability (EGCG 99.7 ± 5.3%, H_2O_2 94.0 ± 1.1%), whereas the treatment for more than 3 h resulted in significant cytotocity, by a trypan blue dye exclusion assay (data not shown). In order to see clearly the effect of the pro-oxidant property of EGCG on cytokine mRNA up-regulation, we utilized the following treatment conditions: cells were treated with 100 μM EGCG or 100 μM H_2O_2 for 15, 30 and 60 min, respectively, washed with fresh medium, and additionally incubated for 165, 150, and 120 min, respectively (until total incubation period was 3 h). The treatment of the cells with EGCG or H_2O_2 at 100 μM significantly increased the mRNA expression levels of IL-2, IFN-γ, IL-5 and IL-13 in a time-dependent